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FEEDING AND DIGESTION IN ELASMOBRANCHS: TYING DIET AND PHYSIOLOGY TOGETHER

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Examining the diet of elasmobranchs provides important ecological and physiological information. There are several techniques available for determining the diet of elasmobranchs including stomach content analysis, stable isotope analysis, and fatty acid analysis. Each technique has its own advantages and drawbacks and the aim of the study will determine the choice of technique, along with other technical considerations. Furthermore, diet preference appears to be dependent on both intrinsic and extrinsic factors that can provide insight into elasmobranch physiology. In addition, the diet may ultimately determine the physiology of the gastrointestinal tract itself, from overall activity level to enzyme expression. Anatomically, the elasmobranch gastrointestinal tract consists of several discrete structures and associated organs. Each organ has distinctive functions in digestion and

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elasmobranchs possess several unique organs not found in other species. The digestion of consumed food proceeds through an assortment of digestive enzymes and secretions. The secretion of the enzymes is most likely dependent on the diet consumed, while activity levels appear enhanced in elasmobranchs compared to other aquatic ectotherms. Overall, elasmobranchs occupy a number of ecological niches and their species-specific physiology is suited to this spectrum of demands. Future work is needed to expand our knowledge of how the consumption of a variety of diets affects the gastrointestinal tract and elasmobranch physiology.

1. INTRODUCTION

Knowledge of the feeding habits of elasmobranchs has been used for a number of different aims: from studying the natural history and evolution of sharks, to modeling predator–prey interactions in marine ecosystems, to estimating the impact of feeding on commercially valuable prey. Ecologically, dietary information is crucial to assess the role of predators in community structure and dynamics, as well as a larger understanding of the ecosystem that the animals inhabit. Physiologically, as diet compositions show from where animals derive their sustenance, dietary information is crucial to understanding the homeostatic processes occurring in animals, both within the gastrointestinal tract (GIT) as well as throughout the body. Understanding what elasmobranchs eat, how often they eat, and whether their diet changes, will allow a better understanding of the physiology of the GIT observed in these species, as it has in teleosts (e.g., Buddington et al., 1987). While the ability of an elasmobranch to consume a wide array of prey species is a challenge in feeding studies, it does suggest an inherent plasticity or flexibility in GIT function. Interestingly, elasmobranchs possess a relatively anatomically uniform GIT across species despite a broad range in dietary preferences and occupation of ecological niches and trophic levels.

Fig. 6.1 shows a simplified breakdown of possible feeding and digestion scenarios for sharks. First, the type of prey consumed may determine the type of feeding approach that is used, either continuous feeding (i) or intermittent feeding (ii). Subsequently, the type of feeding approach may have implications on the digestive processing in these animals (Fig. 6.1). For example, linear (e.g., Cortés and Gruber, 1990, 1994) and exponential (e.g., Sims et al., 1996) gastric evacuation rates have been observed in several types of elasmobranchs and could reflect continuous and intermittent feeding respectively (Fig. 6.1). Additionally, in order to digest different prey items efficiently, the type of enzymes expressed (and/or their

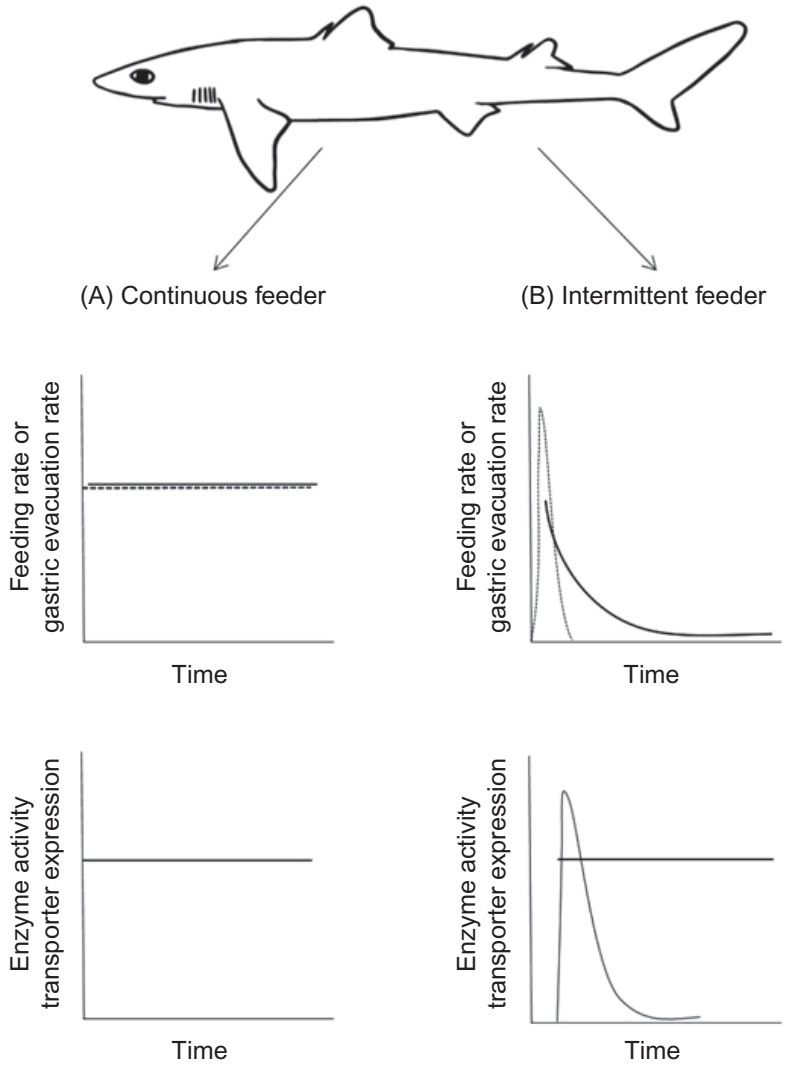

Figure 6.1. Potential elasmobranch feeding ecology and subsequent gastrointestinal tract physiology. Animals may either (A) continuously consume food (dashed line) and evacuate digesta from the stomach (solid line) or (B) consume individual meals infrequently (dotted line) and evacuate digesta from the stomach in an exponential rate (solid line). The consequences of the feeding strategy on the gastrointestinal tract physiology (for example enzyme activities, transporter expression, etc.) could reflect meal consumption frequency.

activity level) in the GIT may depend on both the length of time available for digestion, as well as the type of nutrients to be catabolized (Fig. 6.1). There is some evidence that continuous feeders maintain their GIT in a state that is prepared to digest food, whereas intermittent feeders may up- and down-regulate the GIT in response to feeding (e.g., Papastamatiou and Lowe, 2004, 2005; Papastamatiou, et al., 2007). Although not yet shown in

elasmobranchs, there is evidence that teleost fish are able to move between the categories depicted in Fig. 6.1. Hence, prey choice, feeding strategy, and digestive processing could affect GIT physiology in terms of molecular and cellular responses to feeding in order to optimize the digestion process (e.g., Buddington et al., 1987). Therefore, to understand the physiology of the GIT, it is important to know the type of food ingested and the frequency of feeding, as well as any changes in both throughout the life history of sharks.

2. FEEDING HABITS OF ELASMOBRANCHS

Optimal foraging theory (OFT) suggests that predators select the most energetically profitable prey item to maximize fitness (Pyke, 1984; Parker and Smith, 1990). Essentially, OFT predicts that animals maximize energy coming in and minimize energy going out, which results in increased growth, and eventually translates into reproductive success. To use OFT to examine elasmobranch physiology, we must first examine what elasmobranchs are consuming. For most terrestrial animals, simple observation of prey selection can estimate diet composition. However, direct observation of feeding behavior of elasmobranchs presents practical and technical limitations precluding this approach. Traditionally, researchers have identified prey items by examining the contents of GIT, either through morphological or molecular detection. While this approach yields important information, it is just a snapshot in time. Recent approaches, including stable isotope analysis and quantitative fatty acid analysis, rely on the persistent biochemical signature of long-term feeding history.

2.1. Techniques for Studying Elasmobranch Diets

2.1.1. Stomach Content Analysis

Stomach content analysis (SCA) involves terminal sampling of the stomach and subsequent identification of items found therein. Morphological identification of stomach contents is the most standard method found in the literature. In this case, key anatomical traits are used to assess collected stomach contents and identification of the exact prey species is based on observed, defined morphological characteristics (e.g., Barnett et al., 2010). This is the most common approach found throughout literature and has provided most of the knowledge that we possess about elasmobranch diets. Technological advances have made possible the identification of stomach contents based on DNA analysis. This involves the development of universal primers that amplify the DNA of a wide range

of taxa. These primers are then used against the pooled DNA of all prey collected from the stomach of sampled animals. Subsequently, the observed prey DNA sequences are referenced to known sequences, identifying the species present in the stomach. More specific primers can be developed that are focused on specific groups (i.e., invertebrates vs. vertebrates or a specific class of animals; e.g., Deagle et al., 2007; Valentini et al., 2008), which requires previous knowledge of the prey species likely to be encountered. Using this approach with elasmobranchs has resulted in a 90–100% accuracy rate in prey species identification (Barnett et al., 2010; Sigler et al., 2006; Dunn et al., 2010); however, it is not currently a common approach.

Both approaches have advantages and disadvantages. The simplistic, inexpensive nature and straightforwardness of morphological identification are advantages of the approach (Table 6.1). Rapid identification carried out at the site of collection also obviates sample storage and transport concerns (Table 6.1). Beyond prey identification, morphological identification can also determine the relative proportions of prey types (Table 6.1; see Section 2.1.1.1). However, accurate identification of prey species based on morphology requires extensive expertise in aquatic animal morphology, from zooplankton to mammals, especially when omnivorous predators are examined (Table 6.1). Additionally, this approach often fails to achieve species-level identification, as key diagnostic features may be lost as tissues are broken down through mechanical and chemical digestion (Table 6.1; e.g., Barnett et al., 2010). A lack of hard remains from prey and/or soft bodied prey also may reduce the ability to identify species in stomach contents (e.g., Reñones et al., 2002), and evaluation of diet composition based on the percentage occurrence of prey in the stomach may be biased by differential rates of prey digestion (Table 6.1; e.g., Berg, 1979).

The primary advantage of a DNA-based approach is an improved taxonomic resolution (Table 6.1). Indeed previous studies show that prey DNA identification provides anywhere from a 25–100% increase in specific prey species identification relative to morphological approaches (Barnett et al., 2010; Sigler et al., 2006; Dunn et al., 2010). Another advantage of DNA identification over morphology-based methods is an increase in speed of data collection and generation (Table 6.1; Dunn et al., 2010). While offering a possible improvement over morphological approaches in the level of prey identification, a few disadvantages are specific to DNA approaches. This technique depends on the ability to match the prey DNA sequences against known sequences, which depends on the completeness of DNA databases (Table 6.1). Another disadvantage to this approach is that it requires relatively expensive tissue-specific extraction and analysis (Table 6.1; Deagle and Tollit, 2007). Recent advances in high-through-put sequencing techniques, which allow DNA identification of numerous taxa in large

Table 6.1
Advantages and disadvantages of various techniques for determining the diet consumed by elasmobranchs

Technique	Advantage	Disadvantage
Stomach content analysis		
1. Morphological Identification	Inexpensive	Requires extensive expertise in marine animal morphology
	Rapid	Identifying characteristics may be affected by digestion
	Does not require advanced technology	Difficult to identify prey that lack hard remains
	Can account for relative proportions as long as recently consumed	Differential rates of prey digestion will create bias
		Only identifies recently consumed items
2. Molecular Identification	Highly accurate for specific prey identification	Relies on complete DNA database
	Rapid data collection and preservation	Can be expensive
		Does not account for relative proportion of consumed items
		Only identifies recently consumed items
Stable isotope analysis		
	Can reveal long and short term dietary sources	Low taxonomic resolution
	Can reveal geographic information	Rapid dietary shifts can create unreliable information due to slow tissue incorporation
	Can reveal ontogenetic dietary shifts	Unknown influence on signature by physiology
	Does not have to be invasive or destructive and only requires small tissue sample	Lipid interference
		Accurate quantification via modeling is dependent on parameters that are often unknown for elasmobranchs
Quantitative fatty acid signature analysis		
	Does not have to be invasive or destructive and only requires small tissue sample	Limited experimental knowledge of fatty acid incorporation in elasmobranchs
	May reveal more species-specific level of identification over SIA	
	Can reveal long and short term dietary sources	

numbers (reviewed by Murray et al., 2011), may further increase the speed of the technique, but also the cost of the approach (Table 6.1). Finally, it is difficult to determine the amount of a prey consumed based on DNA, and this approach is more reflective of presence/absence information (Table 6.1).

A shared disadvantage between the two approaches is that SCA only assess recently consumed prey, providing no information on the long-term dietary preferences of animals, or ontogenetic or seasonal shifts in diet (Table 6.1). Cautious interpretation of these studies is required, as diet appears to shift with age, season, animal size, and geographic location, as described shortly.

2.1.1.1. Calculating dietary importance. There is a variety of approaches in quantitatively analyzing the diet based on collected stomach content prey identifications, each with their own advantages. More traditional, basic measurements of counts of individual prey items (numerical quantification of prey items identified in an individual stomach), frequency of occurrence (i.e., the proportion of stomachs containing a specific prey category), and prey volume (weight of individual prey items in a stomach) each reveal specific aspects of dietary information. Prey item counts are representative of immediate feeding behavior, while frequency of occurrence of prey observed across animals sampled reveals population-wide dietary habits, and volume or weight measurements reveals nutritional value of prey consumed. As a more accurate representation of the importance of individual prey items to the diet of predators, Cortés (1997) proposed a composite measure [the Index of Relative Importance (%IRI)], which incorporates the frequency of occurrence, the volume of prey consumed, and the numerical abundance of individual prey items. However, the feeding approach (continuous or intermittent), prey preference, prey size, and gastric evacuation rate all influence the performance of both basic and composition indices of diet analysis (Ahlbeck et al., 2012). Indeed, the analysis of continuously feeding predators and intermittently feeders (Fig. 6.1) showed biases in each analytical approach for estimating dietary importance, especially when examining specialized piscivores (Ahlbeck et al., 2012).

2.1.2. Stable Isotope Analysis

Stable isotope analysis (SIA) is commonly employed to infer diet and trophic relationships within ecosystems and to reconstruct animal diets. SIA involves measuring ratios of heavier and lighter isotopes in animal tissue, and comparing it to the ratio of the particular isotope in an international standard (reviewed by Post, 2002; Martínez del Rio and Wolf, 2005). Generated mathematical models then predict which prey contributed to the observed isotope signature in the predator.

However, observed isotope signatures are heavily dependent on the physiology of the organism and often do not directly reflect those seen in prey. These effects are dependent on both intrinsic and extrinsic factors. Firstly, distinct metabolic pathways determine differential isotope incorporation rates (Martínez del Rio and Wolf, 2005; Ben-David and Flaherty, 2012), a process that is dependent on specific enzyme activities. For example, the oxidation of pyruvate to acetyl coenzyme A affects carbon isotopes incorporation (Martínez del Rio and Wolf, 2005), and elasmobranch tissues or animals with increased pyruvate oxidation (e.g., Treberg et al., 2003) may display altered carbon isotope signatures. Other physiological factors such as animal size, age, stress, growth rate, and nutritional status can affect isotope incorporation rates (Sweeting et al., 2007a,b; Trudel et al., 2011; Weidel et al., 2011) in possibly tissue-specific manners (Caut et al., 2009; Martínez del Rio et al., 2009). Finally, the nitrogen excretion strategy employed by the predator (Minagawa and Wada, 1984; Vanderklift and Ponsard, 2003) can affect isotope signatures. Elasmobranchs are ureolytic, relying on a modified enzyme pathway to produce urea for osmoregulation (Ballantyne, 1997; Hazon et al., 2003).The resulting high urea levels in elasmobranch tissues require a modification of the SIA technique to avoid biasing the findings (Kim and Koch, 2012). External environmental factors such as temperature (Logan and Lutcavage, 2010; Bosley et al., 2002; Trudel et al., 2010) affect isotope signatures as well. Finally, though there are few euryhaline species of elasmobranchs (Wosnick and Freire, 2013), analysis of their diet would require consideration of the potential impact of changes in salinity, which is known to affect SIA (Caut et al., 2009).

SIA has been increasingly used in studies on elasmobranchs (e.g., Papastamatiou et al., 2010; Hussey et al., 2010b; Borrell et al., 2010, 2011; Matich et al., 2011; Speed et al., 2012; Kim et al., 2012a,b) to reveal important information about trophic level position within communities. These trophic levels often match those predicted by SCA (e.g., Cortés, 1999), which reveals the potential to replace this older approach. However, caution is needed because of a poor understanding of isotope incorporation in elasmobranchs (Hussey et al., 2010a) as well as a lack of baseline data (Post, 2002). Indeed, to date there have been few experimental studies to examine elasmobranch-specific isotope incorporation factors (Hussey et al., 2010b; Kim et al., 2012a,b). Existing evidence supports the use of elasmobranch-specific incorporation factors as rates of isotopes were slower (Kim et al., 2012b) than those observed in other aquatic ectotherms (MacAvoy et al., 2006; MacNeil et al., 2006; Logan and Lutcavage, 2010). Additionally, observed incorporation rates varied between tissues and individuals (Kim et al., 2012b). These results suggest that to create accurate

mathematical modeling of isotope incorporation in elasmobranchs, more research is needed on these animals.

SIA has several advantages over SCA. Isotopic ratios are representative of the ratios present at the time of tissue synthesis (Hobson and Clark, 1992) and depending on tissue chosen, SIA can be used to study the temporal variation of diet and habitat use in animals by exploiting tissues with different turnover rates (Table 6.1; Dalerum and Angerbjörn, 2005). Comparing tissues with varying rates of regeneration could offer an opportunity to generate information about dietary shifts throughout the animal's life history, and to construct migratory maps of animals without the need to recapture them later (Table 6.1; e.g., Sweeting et al., 2005; Martínez del Rio and Carleton, 2012). An interesting aspect to SIA is comparing the signatures of animals at various ages, thus revealing ontogenetic dietary shifts with growth (Borrell et al., 2011; Speed et al., 2012) or variability in migration and residency patterns that result in dietary shifts (Papastamatiou et al., 2010). The technique also offers a nondestructive method of tissue sampling (Table 6.1; e.g., blood collection, scale or teeth analysis, muscle biopsy).

Disadvantages of SIA in elasmobranchs have implications for the utility of the approach. There is low taxonomic resolution with this technique and often trophic level estimations are the only result of SIA (Table 6.1; e.g., Matich et al., 2011; Speed et al., 2012). As well, elasmobranchs can have a slow stable isotope turnover rate depending on which tissue is examined (Logan and Lutcavage, 2010). Hence, measurements may miss recent dietary shifts and studying stable isotope incorporation in the lab is difficult (Table 6.1). As outlined above, the physiology of the animal influences isotope signatures and without experimental support, extrapolations from other species may adversely affect the formed conclusions. For elasmobranch study specifically, lipids in particular interfere with isotope measurements (Post et al., 2007; Murry et al., 2006) and therefore must be chemically extracted from the tissue before analysis (Sweeting et al., 2006; Post et al., 2007; Logan et al., 2008). Some shark tissues such as the liver, which are particularly rich in lipids (e.g., Pethybridge et al., 2014), exhibit a known analytical bias when measuring stable isotopes (Table 6.1; Hussey et al., 2010b; Kim and Koch, 2012).

In summary, there are a number of parameters that must be chosen to accurately reflect variables, such as predator physiology or tissue composition (Martínez del Rio and Wolf, 2005; Moore and Semmens, 2008; Parnell et al., 2010; Kim and Koch, 2012), when building a model for SIA. Without experimental supporting evidence, estimates are used to construct these mathematical models (Table 6.1; Phillips and Gregg, 2001, 2003; Moore and Semmens, 2008) and they may not accurately predict diet composition.

There is some evidence that SIA and SCA studies do not agree (i.e., Kim et al., 2012b vs Cortés, 1999) and these differences may result from time of sampling, tissue choice, and/or incorrect models or isotope incorporation factors calculated for SIA.

2.1.3. Quantitative Fatty Acid Signature Analysis

A final approach to determine prey selection in elasmobranchs is the use of fatty acid (FA) signatures (Iverson et al., 2004; Iverson, 2009). FAs are the main component of most lipids and during digestion are released from ingested lipid molecules and are often absorbed into the circulation and then into cells intact (Iverson et al., 2004). Additionally, as marine vertebrates do not synthesize n-3 and n-6 long-chain polyunsaturated fatty acids (PUFAs), integration through the diet is required and thus can act as biochemical indicators of food webs (Tocher and Ghioni, 1999; Dalsgaard et al., 2003; Iverson et al., 2004; Thiemann et al., 2008). This has led to the use of quantitative fatty acid signature analysis (QFASA) to investigate the lipid composition of predators and to reveal dietary prey composition, much like SIA.

In most marine vertebrates, lipids are stored in the adipose tissue typically located in muscle or blubber (Budge et al., 2011). However, elasmobranchs oxidize FAs obtained from lipids in the liver (Moyes et al., 1990; Ballantyne, 1997). Interestingly, though elasmobranchs typically lack adipose tissue in their muscle, several studies have shown the presence of dietary FAs in this tissue (Pethybridge et al., 2010, 2011, 2014). As with SIA, the basis of this technique requires a knowledge of FA signatures in the prey, an understanding of how FA are incorporated into tissues during digestion and assimilation, predator tissue sampling, and a mathematical model to analyze the predator FA signature into the appropriate prey species composition. QFASAs have recently become more popular in chondrichthyan research (Pethybridge et al., 2010, 2011, 2014; Beckmann et al., 2013, 2014). Importantly, initial controlled laboratory research has shown that both liver and muscle FA profiles are indicative of dietary shifts (Beckmann et al., 2014).

There are several advantages of QFASA. Both, SIA and QFASA have the advantage of only requiring a small amount of tissue for analysis, obtained as a biopsy from living animals (Table 6.1). Like SIA, it might be possible to identify changes in diet by examining tissues with differential incorporation rates (Table 6.1; Beckmann et al., 2013, 2014). Additionally, evidence suggests that QFASA may reveal more species-level specificity compared to higher trophic level identification through SIA (Table 6.1; Budge et al., 2002). Indeed, identification of unique FA patterns in marine

fish and invertebrates potentially allows for individual prey species identification (e.g., Iverson et al., 2002; Budge et al., 2002).

However, there has been limited experimental investigation into how prey selection influences the FA profiles of different predator tissues in a controlled environment (Table 6.1). There is also limited information on how the physiology of the predator affects FA incorporation in a tissue specific manner, as is the case with SIA (Table 6.1). For example, the FA profiles of Port Jackson sharks (*Heterodontus portusjacksoni*) fed different diets were indistinguishable in liver but were apparent in the muscle (Beckmann et al., 2013). This may reflect tissue-specific differences in processing lipids, or that the timescale of that study was not adequate to observe the integration of the FA into liver tissue (Beckmann et al., 2014). Future experimentation is needed to investigate temporal FA integration into various tissues, under a variety of environmental constraints, and across life stages. Without these experimental controls, conclusions reached using QFASA may have to be viewed with caution.

Ultimately, combining complimentary biochemical methods such as QFASA and SIA, possibly along with mechanical methods such as SCA, is likely to give broad, useful modeling information. Using these techniques synergistically creates resolutions in consumed prey across varying time-scales, and provides a picture of both current and historical dietary information (Couturier et al., 2013; Connan et al., 2014). QFASA and SIA together also provide important behavioral data, which in turn provides important physiological data, especially if the animal encounters variable environments (Couturier et al., 2013).

2.2. Elasmobranch Diet Composition and Dietary Shifts

The combination of techniques discussed above reveals surprising flexibility in some species and constraints in others. The most familiar elasmobranchs are the apex predators, but elasmobranchs collectively include both generalists and specialists, live in a wide range of habitats, and consume diverse prey (Fig. 6.2). Unfortunately, a number of elasmobranch species do not fit the strict definitions of generalists and specialists. Instead, they appear to exist on a continuum between generalists and specialists both in regards to diet and/or niche occupation (Fig. 6.2; Munroe et al., 2014). To illustrate the problem of categorizing elasmobranchs, a shark can exhibit a far-reaching, global habitat range but be restricted to a narrow range of prey item selection such as the whale (*Rhincodon typus*) or basking (*Cetorhinus maximus*) sharks (Fig. 6.1; Munroe et al., 2014). In contrast, there are sharks that have narrow habitat ranges and wide prey selectivity such as the black tip reef shark (*Carcharhinus melanopterus*; Fig. 6.2;

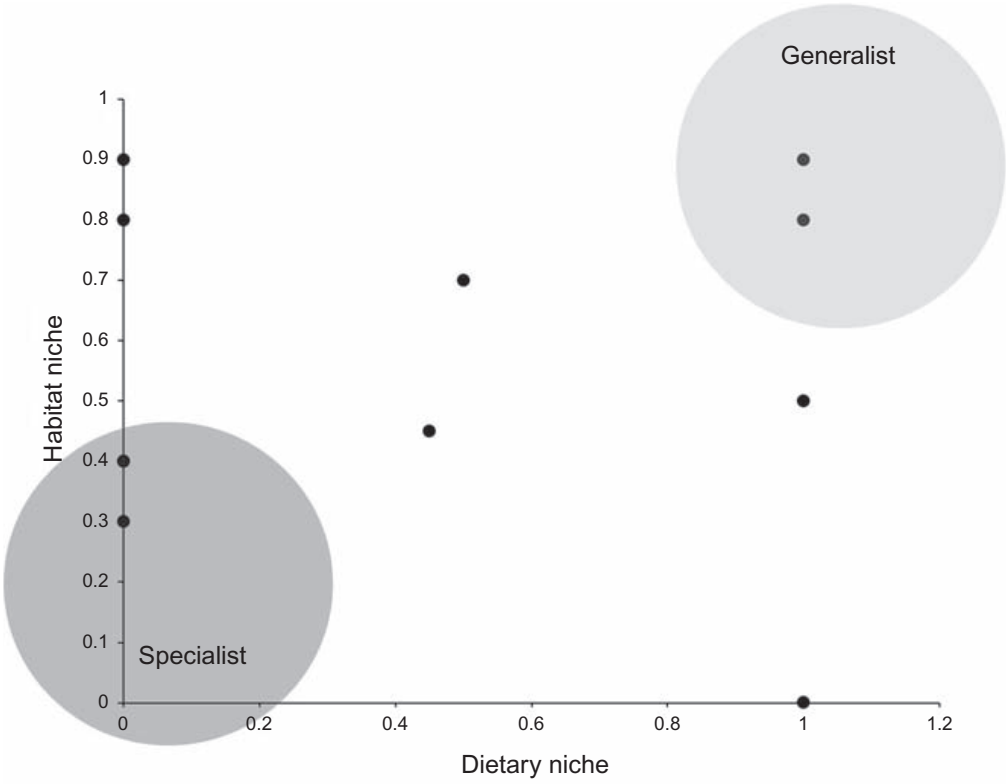

Figure 6.2. Dietary niche and corresponding habitat niches of several elasmobranch species. Dietary niche is defined as the total number of different classes of prey species consumed, with 0 being a single prey species class consumed and 1 being prey consumed from 11 different classes. Habitat niche is defined on a scale of global range with 0 being almost no movement of an individual from the birth location, and 1 being a capability of wide global movement of an individual away from the birth location. A specialist is defined as an individual with low range in habitat and number of species consumed, while a generalist is defined as have a large scope for habitat and diet change. Data points represent individual species of sharks. Data compiled from Cortés (1999), Belleggia et al. (2008), Hoffmayer et al. (2014), and Munroe et al. (2014). Example shark species in the figure include the dusky shark *(Carcharhinus obscurus),* whale shark *(Rhincodon typus),* great white shark *(Carcharodon carcharias),* tiger shark *(Galeocerdo cuvier),* bull shark *(Carcharhinus leucas),* white tipped reef shark *(Triaenodon obesus),* gray reef shark *(Carcharhinus amblyrhynchos),* black tip reef shark *(Carcharhinus melanopterus),* porbeagal shark *(Lamna nasus),* and broadnose skate *(Bathyraja brachyurops).*

Munroe et al., 2014). There are also a variety of feeding behaviors present in elasmobranchs, including selective (feeding on prey in a disproportionate manner to their availability) and opportunistic (feeding on prey in a proportionate manner to their availability) feeding behaviors. An elasmobranch can be characterized as an opportunistic specialist – where the narrow type of diet that is selected is the most available, while another can be a selective generalist – choosing a wide assortment of individual prey

items that may be proportionally rarer compared to other species in the area. As discussed later in the current chapter, there are physiological implications for dietary choice and specialization in regards to intestinal physiology, which reveals the need to define the paradigms used in elasmobranch biology.

In 1999, Cortés compiled a list of sharks, their diet composition, and inferred trophic levels. A quick scan of the compiled list provides examples of loosely defined specialists and generalists, while trophic levels range from 3.1 to 4.7. Compilations on skates (Ebert and Bizzarro, 2009) and rays (Jacobsen and Bennett, 2013) likewise revealed diverse foraging strategies, with specialists and generalists present and similar trophic levels occupied compared to sharks. Ultimately, these studies show the failings of broadly characterizing elasmobranchs as apex predators, and illustrate the breadth of prey and foraging strategies present (Fig. 6.2). Understanding resource use of elasmobranchs provides information critical for successful management solutions for declining populations, as well as for ecosystem protection through understanding community structure. Physiologists can use information of dietary preferences and habitat use (depth, temperature, salinity, etc.) to predict and investigate GIT physiology as discussed below.

2.2.1. Carnivores

The majority of elasmobranch species are classified as carnivores, and there is no record of any plankton or plant material in stomach contents being detected. These predators only consume other animals such as cephalopods, crustaceans, fish, elasmobranchs, or aquatic mammals and birds (Cortés, 1999; Ebert and Bizzarro, 2009; Wetherbee et al., 2012; Jacobsen and Bennett, 2013). Cephalopods are a common prey item of many elasmobranch species. For example, only 21 species of shark failed to consume cephalopods of the 149 species examined by Cortés (1999). A few species of carnivorous elasmobranchs appear to specialize on certain prey items, defined here as $>80\%$ of prey items consumed. For example, *Mustelus californicus* preys almost exclusively on crustaceans, and 19% sharks species noted in Cortés (1999) specialize in preying on teleost fish species. There are also generalist elasmobranch predators that consume a variety of prey animal resources (Wetherbee et al., 2012); however, they may not conform to the strict definition of generalist and may have relatively narrow ecological niche occupancies (Fig. 6.2). It is not clear if this feeding approach reflects an opportunistic feeding pattern as prey densities are rarely co-examined, but the breadth of prey consumed certainly suggests a flexibility in prey capture and digestion.

2.2.2. Planktivores

Filter-feeding of plankton is found in three separate orders: the Myliobatiformes, the Lamniformes, and the Orectolobiformes. Mobulidae (compromised of two recognized species of manta rays and nine species of devil rays) are zooplanktivorous elasmobranchs as indicated by studies using SCA (Notarbartolo-di-Sciara, 1988; Celona, 2004) and SIA (Sampson et al., 2010; Borrell et al., 2010). These species feed on a variety of euphausiids and mysids, with some evidence that they have the flexibility to shift diets between the two depending on the dominant prey in the particular feeding location suggesting they are opportunistic predators (SCA; Notarbartolo-di-Sciara, 1988). However, there is evidence that at least one ray species (*Mobulae japonica*) specializes in a single euphausiid species (SCA and SIA; Sampson et al., 2010). Several other elasmobranch species are planktivores including whale sharks (Gudger, 1941; Motta et al., 2010), basking sharks (Sims, 1999), and the megamouth shark (*Megachasma pelagios*; Nakaya et al., 2008). There is evidence that basking sharks may be specialist feeders, optimally foraging on *Calanus helgolandicus* (Sims and Merrett, 1997). These large planktivores are generally thought to be continuous feeders (Fig. 6.1), feeding regularly and often to meet their metabolic demands with such small prey, although this is mostly speculative knowledge based on animal tracking data using global positioning system (GPS) tags, and correlations with zooplankton availabilities (Anderson et al., 2011; Braun et al., 2014). Sims (2008) estimated that a basking shark consumes 30 kg d^{-1} of plankton.

2.2.3. Omnivores

Only a few species of sharks can be classified as true omnivores, consuming prey across phyla from plants to invertebrates to vertebrates (Cortés, 1999). Bethea et al. (2007) detected large quantities of plant material in the stomach of bonnethead sharks (*Sphyrna tiburo*) representing between 15 and 62% IRI. No experiments were conducted to reveal if the bonnethead shark GIT contains the enzymes required for the digestion of plant material, such as cellulases, and the role of the plant material detected in these animals is not clear.

2.2.4. Dietary Shifts

Shifts in prey species found in the GIT represent a challenge for elasmobranch feeding studies, but also produce good physiological models to study on the effect of diet on GIT function. For example, when a lemon shark (*Negaprion brevirostris*) shifts from crustaceans to fish as the main prey source (Newman et al., 2012), this likely requires a compensation such

as differential enzyme activity patterns in the GIT, as is seen in teleosts (e.g., Chakrabarti et al., 1995; Hidalgo et al., 1999; Drewe et al., 2004). Certainly, chitinase activity would likely diminish with a reduced need to extract nutrients from chitin exoskeletons.

Intrinsic factors such as individual variation and ontological influences can cause dietary shifts. Ontogenetic shifts in elasmobranch diets are commonly observed (e.g., Bethea et al., 2007; Polo Silva et al., 2013; Newman et al., 2012) and are often attributed to the ability to catch larger prey increasing with increasing body size (e.g., Lucifora et al., 2009; Newman et al., 2012) which may increase foraging profitability. This energetic benefit of feeding on large prey is consistent with studies based on OFT of other fish species (Werner and Gilliam, 1984). Baremore et al. (2010) showed that Atlantic angel sharks (*Squatina dumeril*) exhibited prey size selection in accordance with their own gape width. As these sharks consume prey whole (Fouts and Nelson, 1999), this represent the functional limitations of prey size for consumption (Gill, 2003). Ontological shifts can also be caused by changes in habitat occupation and encountered prey communities (e.g., Bethea et al., 2007, 2011; Barbini and Lucifora, 2011). Ontological shifts in diet can change a more generalist juvenile animal (with a broad dietary niche) to a more specialized adult animal (with a smaller dietary niche; Belleggia et al., 2008), further complicating the precise classification of animals (Fig. 6.2).

Extrinsic factors such as geographic and seasonal changes in prey can also create variation in prey consumed by elasmobranchs. For example, the diet of the spiny dogfish (*Squalus acanthias*) in New Zealand is primarily crustaceans (SCA: Hanchet, 1991) while spiny dogfish found in Patagonian waters primarily consume squid (SCA: Alonso et al., 2002), although this had changed dramatically from historical data where hake was the primary prey, illustrating the flexibility of the dogfish to exploit various prey sources. In contrast, spiny dogfish off the West Coast of Canada fed mainly on teleosts in the winter and invertebrates in the summer (Jones and Green, 1977). This regional specification in prey consumption has also been observed for the sandbar shark (*Carcharhinus plumbeus*; McElroy et al., 2006; Cliff et al., 1988; Medved et al., 1985). These differences most likely reflect differences in habitat associated with different geographic locations (e.g., seagrass beds vs. hard bottom substrates) as seen in the bonnethead shark (Bethea et al., 2007).

Ultimately, prey selection appears to depend on ontogeny, geographic location, season, and prey abundance and it is difficult to predict which factor, or combinations of factors, will be important for each species of elasmobranch. Belleggia et al. (2008) showed that ontological shifts in diet selection were present while differences in geographic location showed little

influence on prey selection in the broad nose skate *(Bathyraja brachyurops)*. In contrast, the Australian weasel shark *(Hemigaleus australiensis)* showed a significant influence of ontogeny and geographic location (Taylor and Bennett, 2008) while the bonnethead shark showed ontological differences only in certain geographic locations (Bethea et al., 2007). The spotback skate *(Atlantoraja castelnaui)* showed changes in prey selection with ontogeny, shifting from decopods to teleosts and elasmobranchs with increasing size, seasonal and regional changes in prey abundance (Barbini and Lucifora, 2012).

Regardless, shifts in prey preference and size selection may have significant impacts on GIT physiology. Ontological shifts in prey, for example in the spiny dogfish moving from smaller to larger prey (Bowman et al., 1984), may allow movement between the suggested paradigms in Fig. 6.1. Finally, shifting from energetically lower value animals such as decopods to energetically rich prey such as teleosts, as happens in the spotback skate (Barbini and Lucifora, 2012), will present the intestine with higher nutrient loads, potentially affecting metabolic pathways, transporter expression, and gastric evacuation rates (Sibly, 1981).

2.3. Food Consumption Rates and Gastric Evacuation

OFT is based on the assumption that predators maximize their fitness by selecting their diet to reflect energetically profitable prey. OFT then provides the framework around which to form hypotheses and test theories about prey selection, behavior, and evolution (e.g., Pyke, 1984; Parker and Smith, 1990). OFT includes models of optimal prey digestion which suggest that consuming nutritionally low-quality food necessarily results in ingesting larger amounts of such food and/or developing larger GITs when contrasted with consuming higher quality foods (Sibly, 1981). Optimal prey digestion also predicts that an increasing quality of prey items will result in a decreased transit or digestion time (Sibly, 1981).

In Fig. 6.1, a paradigm is suggested where consumption rates and prey (or ration) size or type may reflect both prey consumed and the physiology of the GIT. Increased feeding rates or decreased ration size in elasmobranchs can increase the speed at which digesta or chyme travels through the GIT (e.g., Meyer and Holland, 2012). Indeed, gastric evacuation time was increased by 50% when meal size was decreased by 8-fold in the scalloped hammerhead shark (*Sphyrna lewini*; Bush and Holland, 2002). Sims et al. (1996) found an inverse correlation between time until appetite return and the gastric evacuation rate in the lesser spotted dogfish *(Scyliorhinus canicula)*, which suggests that as GIT transit times decrease, the more frequently elasmobranchs will feed. The size of the prey items may also

influence gastric evacuation times in elasmobranchs, with larger prey taking longer to digest (Bush and Holland, 2002). Conversely, consuming smaller prey more often will also affect the gastric digestion pattern, increasing feeding frequency and/or digesta passage rates. Taken all together, this logically suggests elasmobranchs that feed on large prey or that ingest large quantities at once will feed less often (intermittent feeders), while those that feed on smaller prey or take smaller meal sizes will be more continuous feeders (Fig. 6.1).

In teleost fish, other factors affect GIT physiology and the rate of digesta processing. The size of the predator itself affects gastric evacuation times, with smaller teleosts evacuating the GIT at a higher relative rate (g digesta g body mass^{-1} h^{-1}) than larger teleosts (e.g., reviewed by Bromley, 1994; Gillum et al., 2012). These findings appear variable and may reflect prey type and/or growth as a confounding factor (e.g., Dunbrack, 1988). There have been no studies conducted on elasmobranchs to determine if this correlation is likewise observed. Digestion of different prey species may occur at different rates in several teleost species and potentially elasmobranchs (Jackson et al., 1987). *In vivo* evidence suggesting that prey with hard exoskeletons take longer to evacuate and digest compared to soft-bodied prey support these *in vitro* studies (Jones, 1974; MacDonald et al., 1982; Bromley, 1991). This is of particular relevance to SCA as differential rates of prey digestion could bias conclusions about diet composition (Table 6.1; e.g., Berg, 1979). Crabs and octopi require a longer digestion time compared to teleost fish in sandbar sharks, increasing digestion time by 20 h (Medved et al., 1985; Medved et al., 1988). Selection of prey sizes may depend on stomach fullness and/or appetite as frillfin gobies (*Bathygobius soporator*) choose prey size inversely proportional to stomach fullness (Tomida et al., 2012). It is unknown if this is also true for elasmobranchs.

The anatomy of the GIT itself may affect gastric evacuation times. In teleost fish, species-specific evacuation times are thought to be partially attributed to narrow sphincters and intestines, restricting the passage of large undigested objects from the stomach (Edwards, 1971; Kionka and Windell, 1972) resulting in a slower evacuation and longer digestion time. A larger intestine diameter may represent an adaptation in increased nutrient assimilation (MacDonald et al., 1982) while increased intestine length may slow digestion times in many herbivores with efficient digestion (Sibly and Calow, 1986; Munoz and Ojeda, 2000; Buckle and Booth, 2009). Wetherbee and Gruber (1993) reported that digestion time in the carnivorous lemon shark was longer than that observed in similar carnivorous teleosts (Wetherbee and Gruber, 1990; Sims et al., 1996; Wetherbee et al., 1990). The protracted time of digesta retention may be related to the shorter intestine in sharks compared to

teleost fish (Section 3.4), which reflects an increase in time needed by sharks to extract nutrients (Sibly, 1981).

Another factor that affects gastric evacuation and digestion time is environmental temperature, inversely decreasing the time it takes to process a meal with increasing temperatures (e.g., Gillum et al., 2012). Additionally, bonnethead sharks show selection for increased ration size with decreasing latitude, suggesting that with increasing water temperatures the increased metabolic demand must be met by increasing energy consumed (Bethea et al., 2007).

More work is needed to address the hypothesized paradigm presented in Fig. 6.1, and to assess the similarity or differences of elasmobranchs with other ectotherms. Nevertheless, species-specific feeding ecology is likely to influence digestive physiology (Fig. 6.1; Secor and Diamond, 1998; Papastamatiou and Lowe, 2005). Feeding frequency is responsible for species-specific differences in the digestive physiology for a number of reptile species (Secor et al., 1994; Secor and Diamond, 1998), and it is possible that this will be true for elasmobranchs as well. Papastamatiou and Lowe (2004) proposed that continuous gastric acid secretion in elasmobranchs is a primitive mechanism to increase the speed of digestion causing a more rapid return of appetite and hence energy consumed (Wetherbee et al., 1990; Sims et al., 1996). In fact, Papastamatiou (2007) predicted that digestion time decreased by $\sim$6 h in the leopard shark *(Triakis semifasciata)* when the stomach was already acidic prior to meal consumption. This may be advantageous for animals that consume resources that are unpredictably available and/or are opportunistic or continuous feeders. They further hypothesize that maintaining gastric acid secretion is energetically efficient compared to down-regulation and then subsequent resynthesis and expression of transporters, proteins etc. (Papastamatiou and Lowe, 2005), much like the state of readiness observed in the intestines of snakes that feed regularly (Secor et al., 1994). It would be interesting to test this hypothesis with other continuously feeding shark species such as the lemon, sandbar, and scalloped hammerhead (Medved et al., 1985; Cortés and Gruber, 1990; Bush and Holland, 2002). In contrast a sporadic feeder, such has been suggested for the spiny dogfish (Jones and Green, 1977; Tanasichuk et al., 1991; Wood et al., 2005), may have GIT physiology closer resembling an intermittently feeding teleost or possible a sporadically feeding reptile (e.g., Secor and Diamond, 1998) with a large scope for changes in intestinal physiology during feeding and fasting.

Feeding frequency as cautiously inferred from the proportion of animals caught with empty stomachs, suggests that bonnethead sharks feed rather continuously while animals like the blacktip shark (*Carcharhinus limbatus*), the finetooth shark *(Carcharhinus isodon),* the dogfish, and the spinner

shark *(Carcharhinus brevipinna)* are intermittent feeders (Jones and Green, 1977; Bethea et al., 2004, 2007). The infrequency of feeding may depend on diet consumed, which in turn may depend on the environment, or geographical region the animal inhabits (e.g., Bethea et al., 2007). Clearly, a number of factors affect gastric evacuation rates and little experimental evidence exists examining elasmobranchs specifically.

3. ELASMOBRANCH GASTROINTESTINAL TRACT ANATOMY

The basic function of the GIT is to provide an avenue for the ingestion, digestion, and absorption of food and energy through mechanical, chemical, and transport processes. A number of specific anatomical divisions in the GIT, namely the buccal cavity and pharynx, the esophagus, the stomach, and the intestine, are responsible for these processes. There may be associated organs with each of these divisions that may or not be functionally obligated to the process of digestion. The reader is encouraged to pursue the wide assortment of additional reviews on elasmobranch GIT anatomy (Fänge and Grove, 1979; Holmgren and Nilsson, 1999; Cortés et al., 2008).

Teleost fish occupy several ecological niches and trophic levels similar to elasmobranchs. Unlike elasmobranchs, teleost fish demonstrate a diversity in GIT anatomy (Kapoor et al., 1975; Fänge and Grove, 1979; Wilson and Castro, 2010), having evolved to suit the diet consumed as well as to serve a variety of other functions such as air breathing (e.g., Graham, 1997; Nelson, 2014) and osmoregulation (e.g., Grosell, 2007; Larsen et al., 2014). Numerous studies have investigated the anatomical adaptations of the teleost GIT to various diets (Wilson and Castro, 2010). Most studies reveal that while correlations between diet and anatomical structures are weak, correlations between functional adaptations (i.e., enzyme expression profiles) appear related to the nature of the prey consumed (e.g., Chakrabarti et al., 1995; Hidalgo et al., 1999; Drewe et al., 2004). Unlike teleost fish, which also display a range of dietary preferences, the gross anatomy of the GIT of elasmobranchs is not as reflectively diverse across species. There are several smaller, species-specific variances in the anatomy addressed below, but overall the GIT of elasmobranchs is similar across species.

3.1. The Buccal Cavity, Pharynx, and Associated Structures

The buccal cavity in elasmobranchs represents the entry to the GIT and is associated with the selection and seizure of prey. There are varieties

of morphological buccal adaptations in elasmobranchs that are representative of dietary specializations along with associated morphology of the feeding mechanisms (e.g., Motta and Huber, 2012). Subsequent studies examining the function of the feeding mechanisms reveal much about the prey targeted by the sharks as well as their feeding strategies (e.g., suction feeding, ram feeding, bite feeding) and an excellent review exists (Motta and Huber, 2012) on the functional morphology of the buccal cavity and its association with the rest of the feeding mechanism. The lining of the buccal cavity consists of modified placoid scales in the cow shark (*Heptanchus maculatus*; Daniel, 1934) and lacks glands present in higher vertebrates. The lining of the pharynx consists of stratified cuboidal cells interspersed with mucous glands (Chatchavalvanich et al., 2006). The basking shark pharyngeal epithelium is additionally marked with papillae that may aid in food particle capturing (Matthews and Parker, 1950).

The gills slits are located in the ventrolateral walls of the pharynx through which the respiratory water current reaches the gills for gas exchange. Supporting their adaptations to filter feeding, pharyngeal sieving of food particles has been noted in the Mobulidae (Cortés et al., 2008; Paig-Tran et al., 2013). The Lamniformes (basking sharks and megamouth sharks) and Orectolobiformes (whale sharks) have particularly large and complex sieving plates for filtering and trapping food particles (Cortés et al., 2008; Paig-Tran et al., 2013). Associated with the pharynx are the thymus and thyroid glands (Luer et al., 1995). The associated pharyngeal musculature is similar to that seen in teleost fish (Mallatt, 1997).

3.2. The Esophagus

The pharynx leads directly into the esophagus (Fig. 6.3A and B), often separated by a sphincter that is closed except when passing food. The cells found within the esophagus are typically stratified columnar epithelial cells with abundant mucous cells (Fig. 6.4A; Holmgren and Nilsson, 1999; Chatchavalvanich et al., 2006). Occasionally there are long, finger-like projections into the esophageal lumen (Leake, 1975). The purpose of these projections are not clear.

The Leydig organ is an important esophageal-associated organ in most species of elasmobranchs and constitutes two masses of tissue found along the dorsal and ventral portions of the esophagus (Fig. 6.4A; Mattisson and Fänge, 1982; Li et al., 2013). The role of this unique organ is to aid in the production of red blood cells (also carried out by the spleen as in most other vertebrates) and other lymphoid activities categorizing it as a lymphomyloid

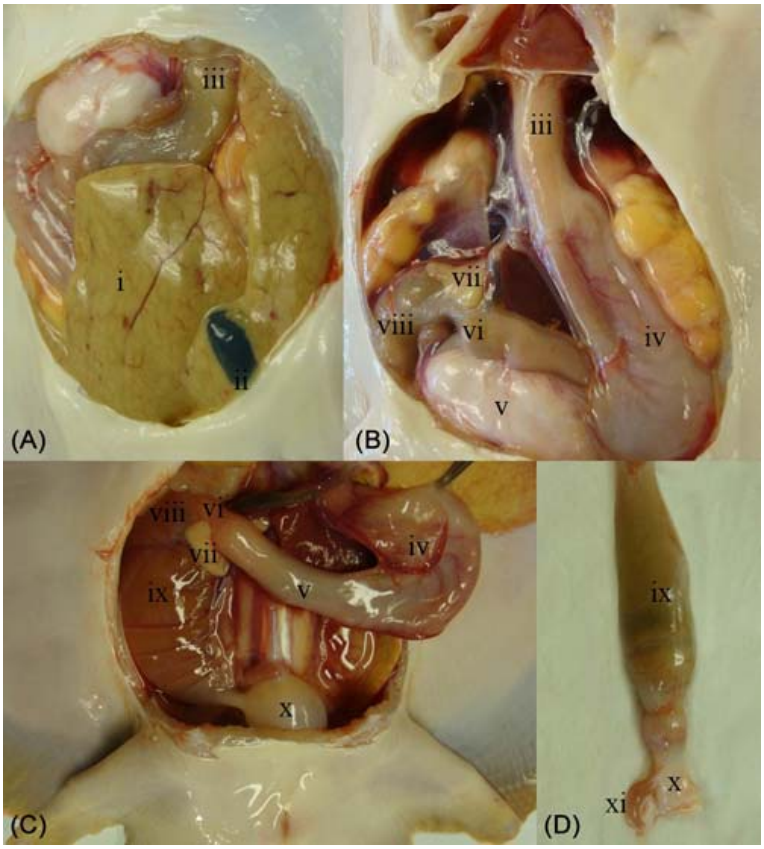

Figure 6.3. The gastrointestinal tract of the skate. (A) Ventral view of liver (i), gallbladder (ii), and esophagus (iii). (B) Ventral view of esophagus (iii; not pictured: Leydig Organ), cardiac stomach (iv), pyloric stomach (v), bursa entiana (vi), pancreas (vii), and anterior intestine (viii). (C) Ventral view of cardiac stomach (iv), pyloric stomach (v), bursa entiana (vi), pancreas (vii), and anterior intestine (viii), spiral intestine (ix), and colon (x). (D) View of spiral intestine (ix), colon (x) and rectal gland (xi) removed from animal. Photos provided by W.G. Anderson.

tissue (Mattisson and Fänge, 1982). According to Mattisson and Fänge (1982), the Leydig organ can be quite large – 1.6 kg of tissue in a cow shark (1.8 m in length) and 1.2 kg of tissue in a Greenland shark (*Somniosus microcephalus*; 2.9 m in length) and can account for 0.5% of total body weight. Early light microscopy and ultrastructural characterization has revealed a similarity between Leydig organ cells and those from the epigonal organ in elasmobranchs as well as those from the mammalian lymphomyeloid systems (Mattisson and Fänge, 1982). Recent investigations using molecular approaches have revealed that the Leydig organ appears to function in conjunction with the thymus and epigonal organs as the primary sites of lymphopoiesus (Anderson et al., 2004; Li et al., 2013).

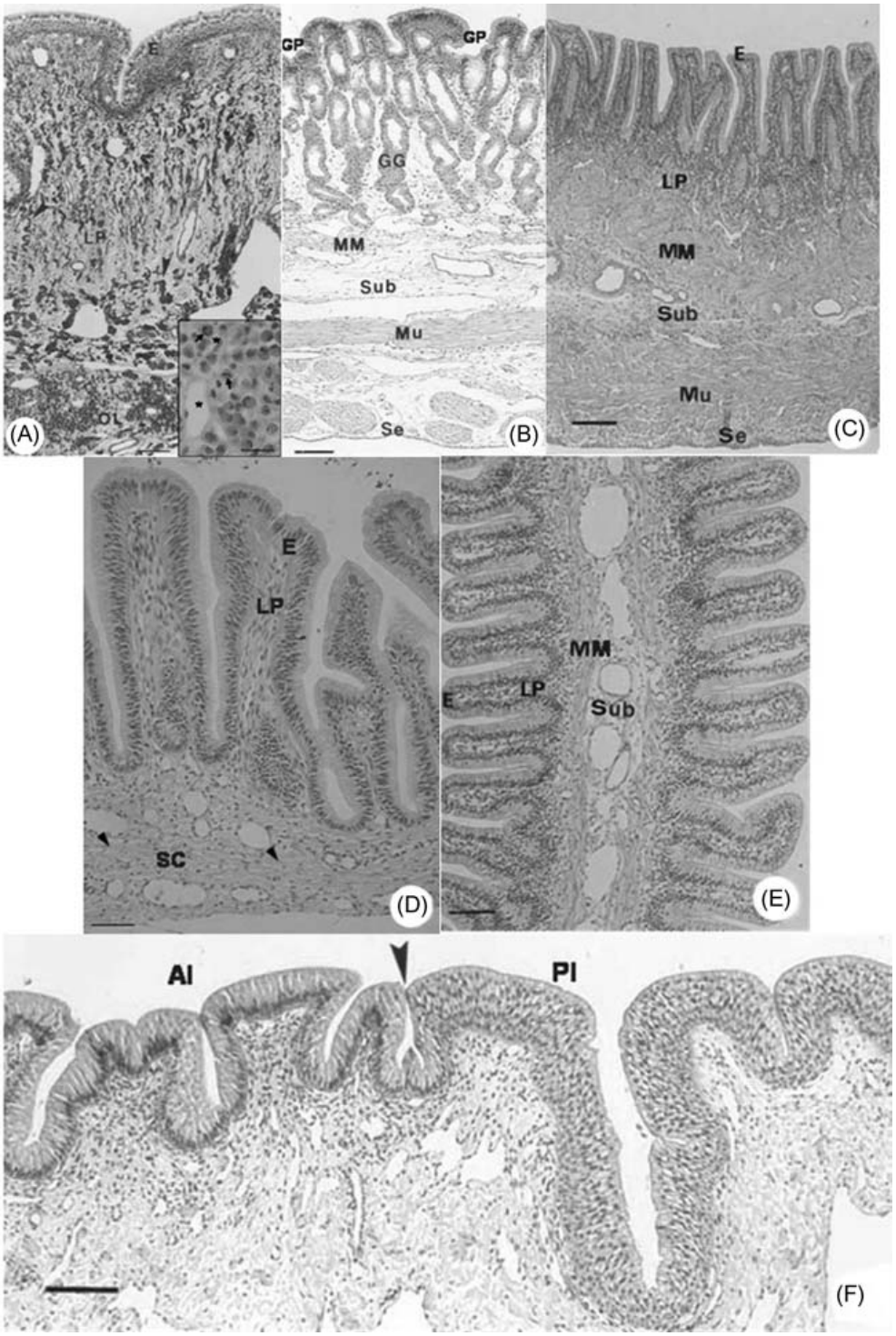

Figure 6.4. Histology of the gastrointestinal tract of the freshwater stingray (*Himantura signifier*). (A) Cross section of the esophagus epithelium (E) and laminia propria (LP). Collagen fibers are present in the LP (arrowhead). Also shown is the organ of Leydig (OL) embedded in the LP; inset shows OL as lymphomyeloid tissue with blast cells (arrows) and leucocytes (asterisk). Bar = 100 μm. Inset: Bar = 25 μm. (B) Cross-section of the cardiac stomach,

3.3. The Stomach

Lying posterior to the esophagus is the stomach. The stomach stores ingested food material and begins the initial chemical and enzymatic breakdown of food during digestion. Typically, it is characterized as a J-shaped or U-shaped organ in a species-specific pattern (Holmgren and Nilsson, 1999). The esophagus connects to the anterior, or descending portion of the stomach, which known as the cardiac portion of the stomach (Fig. 6.3B). In contrast, the posterior, or ascending portion, is known as the pyloric stomach (Fig. 6.3B). The lining of the stomach consists of columnar cells with microvilli (Fig. 6.4B and C; Chatchavalvanich et al., 2006), which not only line the stomach but also make up the gastric glands interspersed in the stomach lining. Interestingly, histochemical characteristics of many of these columnar cells are indicative of absorptive cells (Chatchavalvanich et al., 2006). Columnar cells are also found in teleost stomachs (Grau et al., 1992) and whole animal evidence suggests the stomach is a site of absorption (Bucking and Wood, 2006, 2007). Recently, observed ion absorption in the stomach of the spiny dogfish (Liew et al., 2013) suggests this may be a shared phenomenon.

The gastric glands of elasmobranchs are the sites of digestive fluid secretions, namely HCl and pepsinogen, and are flask-like in shape and consist of a variety of cells (Fig. 6.4C). The current assumption is that lower vertebrates such as fish and sharks have a single type of secretory gland cell in the gastric gland – the oxynticopeptic cell (Fänge and Grove, 1979; Grabowski et al., 1995; Hamlett et al., 1996; Holmgren and Nilsson, 1999). This cell is responsible for the secretion of both HCl and pepsinogen for digestion. This is in contrast with higher vertebrates such as mammals that have separate, distinct cells for each: parietal cells for HCl secretion and chief cells for pepsinogen secretion. This view has been called into question after several studies on elasmobranchs revealed the potential presence of separate parietal and chief cell equivalents in the sixgill shark (*Hexanchus*

showing gastric pits (GP) and gastric glands (GG) in the mucosa. Muscularis mucosae (MM), submucosa (Sub), muscularis (Mu), serosa (Se) also shown. Bar = 100 μm. (C) Cross-section of the pyloric stomach epithelium showing the LP and the thin stratum compactum (SC) (the upper limit of the SC is marked by arrowheads). Bar = 40 μm. Note the lack of gastric glands. (D) Cross section of the anterior intestine epithelium. Bar = 100 μm. Note the numerous mucosal folds. (E) Cross section of the spiral intestine, showing the spiral valve in detail. Bar = 50 μm. (F) Longitudinal section of the anterior–posterior intestine junction (AI versus PI), revealing the abrupt change of epithelium (arrowhead), from simple columnar (at left) to stratified columnar (at right). Bar = 100 μm. These images were modified from Chatchavalvanich et al. (2006). Refer to original manuscript for more details. Modified and reprinted with the generous permission of Spring-Verlag and R. Marcos.

griseus; Michelangeli et al., 1988), the Atlantic stingray (*Dasyatis sabina*; Smolka et al., 1994), and finally the freshwater whip ray (*Himanttura signifier*; Chatchavalvanich et al., 2006). The reason behind this apparent species-specific evolution of gastric cells in elasmobranchs is not currently known.

Interestingly, mammalian parietal cells use secretory canaliculi and a tubulovesicular system for the secretion of HCl, while it appears that the parietal cell equivalent in the sixgill shark is lacking the secretory canaliculi system (Michelangeli et al., 1988). There was no mention as to the presence or absence of the secretory canaliculi system in the other studies on elasmobranchs with seemingly distinct gastric cells; however, tubulovesicles were observed (Smolka et al., 1994; Chatchavalvanich et al., 2006). Additionally in mammalian stomachs, chief cells and parietal cells intermingle spatially in the integument, while at least in the sixgill shark there is a definite zonal separation of the parietal cell equivalents and the chief cell equivalents (Michelangeli et al., 1988). In elasmobranch species where combined oxynticopeptic cells are found, gastric glands may also exhibit zonation, being found primarily in the cardiac and fundic regions, and not the pylorus (Grabowski et al., 1995); suggesting a gradient of proteolytic enzymes and HCl along stomach in either case (Fig. 6.4B vs. C; Chatchavalvanich et al., 2006).

3.4. The Intestine and Associated Structures

Posterior to the stomach is the intestinal portion of the GIT separated from the pyloric stomach by a circular band of muscle fiber forming the pyloric valve or sphincter. In some species there is a small chamber that the pyloric valve opens into, known as the bursa entiana (Fig. 6.3B and C). The purpose of this chamber is unclear, although in the coffin ray *(Hypnos monoterygius)* there is a partition separating the bursa entiana into two further smaller chambers (Daniel, 1934). The intestine is broken down into two sections: the proximal small intestine (or anterior intestine or duodenum), and the distal large intestine (or posterior intestine or spiral intestine; Fig. 6.3B–D). In a few species of elasmobranchs there are one to several large appendages attached to the anterior intestine (Holmgren and Nilsson, 1999) similar to pyloric caecae seen in teleost fish. The function of the caecae in sharks is unclear; however, in teleost they may increase surface area for absorption, and aid in lipid absorption (Buddington and Diamond, 1987) and possibly osmoregulation (Veillette et al., 2005). The epithelial lining of the small intestine consists of simple absorptive columnar cells with microvilli, goblet cells with neutral and acid mucins, and enterochromaffin cells (Fig. 6.4D; Chatchavalvanich et al., 2006).

The epithelium is folded (Fig. 6.4D) presumably to increase surface area of the short anterior intestine.

The anterior intestine is also the location of connections to the liver and pancreas, and hence where biliary and pancreatic secretions occur. The liver is a large, bi-lobed organ that is the location of urea synthesis and amino acid catabolism in elasmobranchs (Fig. 6.3A). These products are of utmost importance in the osmoregulatory and nitrogen balance strategy of elasmobranchs (see Chapters 4 and 5). Associated with the liver is the gall bladder, which is where bile is stored before secretion into the anterior intestine via the biliary duct (Fig. 6.3A). Bile production rates (1–1.5 μl kg^{-1}, min^{-1}; Boyer et al., 1976) are on par with those observed in teleost fish (Grosell et al., 2000).

The pancreas is composed of two compact, discrete, and connected lobes that empty into a common duct connected to the anterior intestine (Fig. 6.3B and C). The pancreas has both exocrine and endocrine functions. The exocrine pancreas produces digestive enzymes such as lipases and colipases (Sternby et al., 1983), and occasionally chitinases typically when insects and crustaceans are a large dietary component (Fänge et al., 1979). Gastric acid additions to the anterior intestine stimulate these secretions (Babkin, 1929, 1932), although limited work has occurred since these early studies. The endocrine pancreatic cells in sharks most likely secrete insulin, glucagon, pancreatic peptide, and somatostatin, as summarized by Holmgren and Nilsson (1999).

The large intestine (Fig. 6.3C and D) is most identifiable by the presence of the spiral valve, although in some species the folds more resemble a scroll (i.e., S. zygaena) (Holmgren and Nilsson, 1999). The spiral valve represents a series of integument folds traversed by blood vessels. The integument of the large intestine consists of simple columnar cells with microvilli, goblet cells with neutral and acid mucins, and enterochromaffin cells as in the small intestine (Fig. 6.4E; Chatchavalvanich et al., 2006). Little work has examined the development of the spiral valve to date. Daniel (1934) suggested that the valve first appears as a ridge along the length of the posterior intestine. This ridge then rolls up into a scroll shape and if subsequent torsion occurs this scroll shape transforms into a spiral shape. The cellular mechanisms at work are unclear and deserve investigation.

The purpose of the spiral valve is likely to increase surface area for absorption and increase digesta transit time, while decreasing the overall size of the intestine (Holmgren and Nilsson, 1999). Indeed, when the length of the intestine is compared to that expected based on scaling with teleost fish (Fig. 6.5), it is obvious that the relative intestinal length is smaller than predicted based diet and species comparisons. However the calculated relative length of the intestine should be interpreted with caution as the body

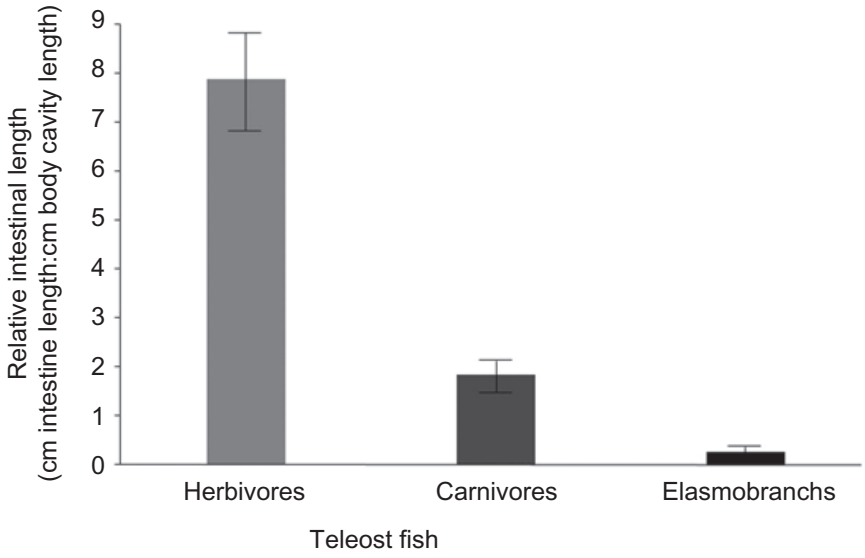

Figure 6.5. The relative length of the intestine compared to the length of the body cavity. Teleost fish are divided into herbivorous and carnivorous fish, while elasmobranchs are grouped together regardless of dietary makeup. Data from personal measurements (C. Bucking, unpublished) or recalculated from Kapoor et al. (1975) for teleost fish and from Holmgren and Nilsson (1999) for elasmobranchs.

cavity of sharks is morphologically distinct from many teleost fish, as it is fusiform to increase swimming efficiency while many teleosts have broader and/or shorter body cavities. Regardless, it is generally accepted that elasmobranch have a shorter than expected intestinal length (Holmgren and Nilsson, 1999). The spiral valve, in theory, slows down the progression of digesta through the intestine, forcing it to wind through the scrolls before elimination. This increase in processing time may allow for sufficient nutrient absorption (Sibly, 1981). One would predict that animals habitually consuming prey items of lower nutrient quality would have a slower transit time and more numbers of spirals. Supporting this is evidence that the number of turns that spiral valves form appears to be species-specific; however, there is evidence of individual variation within a species as well (Holmgren and Nilsson, 1999). The absorptive columnar cells with villi that project into the lumen of the GIT lining this section support the role of the spiral valve in absorption (Chatchavalvanich et al., 2006).

3.5. The Colon, Rectum, and Rectal Gland

The final portion of the GIT is the colon and rectum (Fig. 6.3C and B). The colon is notable for an extensive muscular layer in contrast with the intestine, as well as a transition away from columnar cells with microvilli to

stratified columnar and/or cuboidal cells (Fig. 6.4F; Holmgren and Nilsson, 1999; Chatchavalvanich et al., 2006). The epithelial goblet cells also transition toward sulfated acid mucin staining cells (Chatchavalvanich et al., 2006) and their quantity suggests they aid in passing undigested material out of the GIT. A lumen from the rectal gland demarcates the colon from the rectum. The rectal gland is a digitform organ attached to the GIT and functions in aiding osmoregulation (Fig. 6.3D; Burger and Hess, 1960). The skate rectal gland is physically much smaller than the shark rectal gland, and the secretagogues stimulating chloride secretion differ from those seen in sharks (Kelley et al., 2014). Importantly, the rectal gland function may also be influenced by the process of digestion, with digestion triggering an increase in NaCl secretion (Wood et al., 2008, 2010), and anticipatory transcriptomic responses were recently observed in response to feeding (Deck et al., 2013).

3.6. Ontogeny of the Gastrointestinal Tract

The development of the GIT of elasmobranchs has been restricted to a single study in the southern stingray, *Dasyatis americana* (Hamlett et al., 1996). It appears that the fetal alimentary organs function early in the development of the stingray, to digest and absorb nutrient histotroph produced by the maternal uterus to aid in growth (Hamlett et al., 1985).

4. DIGESTIVE ENZYMES AND SECRETIONS

One of the roles of the GIT is to breakdown incoming food into nutrients for absorption. This is accomplished by a suite of enzymes that may be secreted in distinct portions of the GIT. For example, gastric acid secretion occurs exclusively in the stomach, while bile secretion occurs specifically in the anterior intestine. These enzymes and other secretions are essential for efficient and optimal digestion of prey, and the type of prey consumed or other aspects of feeding ecology often influence their expression.

4.1. Stomach Enzymes and Secretions

The stomach is often the site of the initiation of digestion. It allows the consumption of large quantities of food, forming a holding area where acids and enzymes can begin the process of breaking down ingested material. The stomach then meters out the digesta to the intestine for further digestion.

It is theorized that an acid-secreting stomach first appeared $\sim$350 million years ago in the elasmobranchs (Koelz, 1992). Most vertebrates,

elasmobranchs included, secrete an acidic fluid into the stomach lumen that is primarily made of three components: gastric acid (HCl), acid proteases (pepsinogen/pepsin), and mucous. Through chemical and enzymatic catabolism this fluid aids digestion of prey residing in the stomach. One of the primary secretions associated with the stomach is gastric acid. The cell responsible for gastric acid secretion is either the oxynticopeptic cell in lower vertebrates, or the parietal (oxyntic) cell in mammals. As mentioned above, it appears that several elasmobranch species have also evolved separate acid secreting cells, although their tissue distribution in the gastric mucous appears to follow a distinct pattern compared to the distribution seen in mammals (Michelangeli et al., 1988). Regardless of whether HCl secretion occurs from a distinct cell, the enzyme responsible appears relatively conserved across vertebrates. The H^+, K^+-ATPase is a potassium-stimulated proton translocating adenosine triphosphatase (the HKA). Typically, the HKA is located in tubulovesicle membranes that fuse with secretory canaliculi membranes when gastric acid secretion is stimulated. This exposes the transporter to the stomach lumen where intracellular protons exchange for luminal potassium ions. Chloride channels in the apical membrane of the cells allow Cl^- to enter the lumen and form HCl for digestion. The gastric HKA orthologue in the Atlantic stingray is $>80\%$ identical to the gastric HKA in mammals (Choe et al., 2004), which indicates the evolutionary conservation of this transporter across species (Smolka et al., 1994).

It is clear, that as in other vertebrates, the ingestion of a meal and subsequent digestion triggers an increase in gastric acid secretion in elasmobranchs (Sullivan, 1905). This gastric phase of acid secretion (Olsson and Holmgren, 2011), where the ingestion of food distends and/or raises the pH of the stomach (e.g., Papastamatiou and Lowe, 2004), is further controlled through secretagogues (e.g., gastrin, histamine, and acetylcholine). Evidence of an increase in pH of the stomach and subsequent lowering back to basal values following the ingestion of a meal has been directly observed in elasmobranchs (Papastamatiou and Lowe, 2004; Wood et al., 2005, 2009). Although the mechanism behind the increase is unexplained, the hypothesized mechanism behind the decreasing pH is through increased HKA activity, as a known HKA inhibitor reduced gastric acid secretion (Wood et al., 2009). Acid secretion rates in leopard sharks are pH dependent, with rates of ~ 6 mmol/h when gastric pH was >2.5, and ~ 2 mmol/h when pH was 2.0–2.5 (Papastamatiou, 2007).

There appears to be two approaches for gastric acid secretion in teleost fish: a continuous basal secretion that maintains a low pH in the stomach (e.g., Bucking and Wood, 2009), and a food ingestion trigged release that only lowers the pH away from neutral upon consumption of a meal

(e.g., Nikolopoulou et al., 2011). There is evidence that several species of sharks and rays employ a continuous basal secretion approach (Papastamatiou and Lowe, 2004; Papastamatiou et al., 2007; Wood et al., 2009; Anderson et al., 2010), while Papastamatiou and Lowe (2005) confirmed that nurse shark *(Ginglymostoma cirratum)* had neutral gastric pH when not holding food in the stomach. The two patterns in HCl secretion may relate to feeding patterns as frequent teleost feeders, as well as elasmobranch species that experience unpredictable food availability (Papastamatiou and Lowe, 2004, 2005; Papastamatiou, et al., 2007), tend to maintain a low gastric pH. In contrast, less frequent, sporadic elasmobranch feeders may exhibit a neutral gastric pH between feeding events (Fig. 6.1; Papastamatiou and Lowe, 2005). Yufera et al. (2012) suggests that daily feeding patterns and frequency may control gastric acidification regulation. This results in an ability to move between categories in Fig. 6.1 and represents the adaptability of the GIT to changes in feeding frequency and demands placed on the GIT in order to optimize digestion.

The secretion of gastric acid in elasmobranchs maintains a low pH in the stomach lumen, which likely aids in the activation of the main protease pepsinogen into pepsin, the other notable component of stomach secretions. Pepsinogen secretion is via the oxynticopeptic cells in the gastric mucosa of most nonmammalian vertebrates, but as with gastric acid secretion, there is limited evidence that at least some elasmobranch may have distinct cells (Michelangeli et al., 1988). Gastric acid cleaves pepsinogen into pepsin, creating an active enzyme for protein digestion. Characterization of a pepsinogen in the Portuguese dogfish *(Centroscymnus coelolepis)* revealed a monomeric protein, ~42 kDa in size, with similar characteristics to mammalian proteins (Nguyen et al., 1998), although activity rates of the elasmobranch protein are higher at low temperatures compared to mammalian proteins (Guerard and Le Gal, 1987). Unfortunately, the sequence of pepsinogen is not known in elasmobranchs.

There is evidence of chitinases and high chitinolytic activity in the stomachs of the velvet belly lanternshark *(Etmopterus spinax)* and the thorny skate (*Raja radiate*; Fänge et al., 1976; Fänge et al., 1979), although no follow-up work has been done exploring this. In teleost fish, the presence of chitinolytic activity may aid the digestion of the shells of crustaceans (e.g., Danulat, 1986), which is also likely in elasmobranchs. High activity levels would be predicted in the stomach and intestines of elasmobranch species that prey primarily on crustaceans (Cortés, 1999), although this has not been investigated. Interestingly, initial measurements of chitinolytic activities in the fish GIT were attributed to bacteria. However, additional experiments suggested that the chitinase activities in the digestive tract of Atlantic cod, *Gadus morhua,* were primarily derived from fish tissues (Danulat, 1986).

Recently, three different chitinase genes have been identified in the Japanese flounder *(Paralichthys olivaceus),* two of which were predominantly expressed in gastric glands (Kurokawa et al., 2004). As well, Krogdahl et al. (2005) have provided evidence of chitinase in genome of the pufferfish *(Takifugu rubripes)*. This indicates that teleost fish have the ability to produce endogenous chitinase for digestion and may secrete it with other products of the gastric gland during digestion. While the source of chitinase activity in elasmobranchs remains unknown, what is clear is that relative to teleost fish, gastric chitinase activity is higher (Fig. 6.6A). In fact, chitinase activity in the stomach of elasmobranchs is 100-fold higher than that seen in the stomach of teleosts (Fig. 6.6A; Fänge et al., 1976; Fänge et al., 1979; Danulat, 1986).

4.2. Intestinal Enzymes and Secretions

Once food leaves the stomach, it enters the intestine where nutrients are further broken down and absorbed with water and other factors. The prolonged digesta evacuation time compared to teleost fish may be required for complete breakdown and absorption of nutrients in the shorter than expected intestine (Fig. 6.5). With a shorter intestine, there is less time and/or surface area to breakdown and absorb ingested material; however, the presence of the spiral valve in elasmobranchs most likely serves to compensate for this by increasing surface area. Regardless, this catabolism of nutrients occurs via the secretion of digestive fluids. Lipid metabolism is particularly prominent in elasmobranchs and fat digestion depends on three main intestinal secretions: bile salts and pancreatic lipase and colipase. These secretions also are the most studied in the literature and will be the focus of this section as examples of intestinal enzymes and secretions.

Pancreatic lipase hydrolyses triacylglycerol substrates into diacylglycerol, monoacylglycerol, and free fatty acids; is synthesized in the pancreas; and is secreted into the intestine via a duct. A survey of pancreatic lipase activities in elasmobranchs [*Dasyatis pastinaca* (common stingray), *Mustelus mustelus* (common smooth-hound), *Rhinoptera marginata* (murin), and *Rhinobatos cemiculus* (guitar fish) Smichi et al., 2012] revealed high activities in the sharks compared to other marine animals (Fig. 6.6B). Indeed, pancreatic lipase activity in the pancreas of elasmobranchs is 10- to 100-fold higher than activities observed in equivalent tissues in teleost fish and invertebrates (Smichi et al., 2012). This may reflect the importance of dietary FAs to the energy metabolism of elasmobranchs, as well as increased activity to optimize digestion (Sibly, 1981). It may also reflect the dependence of teleost fish on another enzyme for lipolysis, the bile salt-activated lipase that

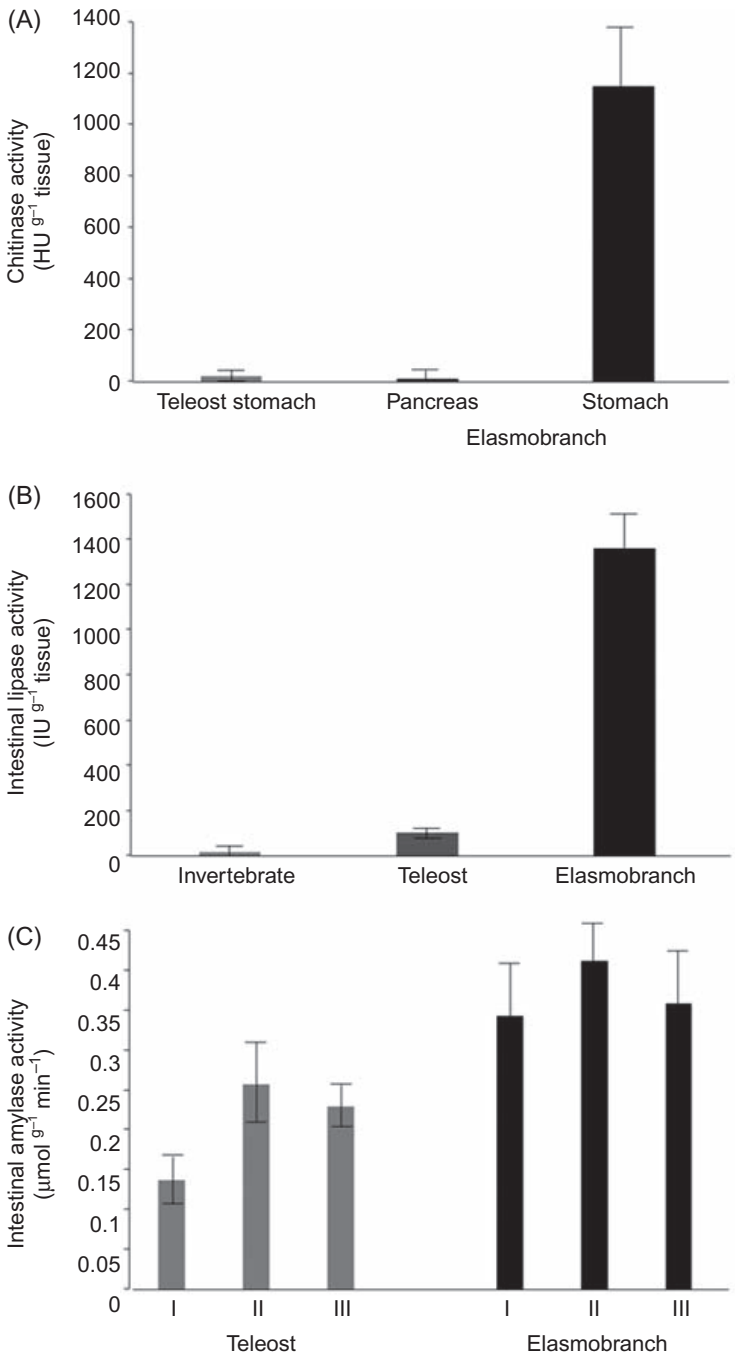

Figure 6.6. Comparative enzyme activities in the gastrointestinal tract and associated structures. (A) Chitinase activity (HU g^{-1} tissue) in the stomach tissue of teleosts and elasmobranchs and the discrete pancreas of elasmobranchs. (HU: Hulten units). Data compiled from Fänge et al. (1976, 1979). Values are means ±S.E. (B) Lipase activity (IU g^{-1} tissue) in the pancreatic (or equivalent) tissues of invertebrates, teleost fish, and elasmobranch fish. Data compiled from Smichi et al. (2012). Values are means ±S.E. (C) Amylase activity (µmol g^{-1} min^{-1}) in the intestinal sections of teleost fish and elasmobranchs. Intestinal sections were broken up from anterior to posterior sections into: proximal (I), middle (II), and distal (III) sections. Teleost data compiled from Chakrabarti et al. (1995), elasmobranch data compiled from Kuz'mina and Gelman (1997).
Values are means ±S.E.

hydrolyses carboxyl ester bonds of acylglycerols, cholesterol esters, and fat-soluble vitamin esters. Unfortunately, limited data exists on species-specific enzyme activity levels in elasmobranchs restricting comparisons. The common smooth-hound, the murin, and the guitar fish display similar pancreatic lipase activity levels, while the activity in the common stingray was an order of magnitude higher (Smichi et al., 2012). Future experiments on different elasmobranch species will reveal any dietary correlations within elasmobranchs and the functional significance of this observation. The pH optimum for elasmobranch lipase activity was between pH 8–9 (Smichi et al., 2012), generally in accordance with the measured pH of the intestine of elasmobranchs (e.g., Anderson et al., 2010).

Elasmobranch pancreatic tissues were positive for the co-enzyme colipase (e.g., Smichi et al., 2012), which optimizes pancreatic lipase activity (e.g., Erlanson-Albertsson, 1992), and colipase was detected in the transcriptome of the lesser spotted catshark (*Scyliorhinus canicula*; Mulley et al., 2014). Further molecular characterization of colipase from the common stingray and dogfish shark revealed a considerable ($\sim$55%) molecular identity of the enzymatically active N-terminal sequence with mammalian colipases (Sternby et al., 1984; Bacha et al., 2011). Analysis of the Atlantic cod, Japanese pufferfish, Japanese rice fish *(Oryzias latipes)*, three-spined stickleback *(Gasterosteus aculeatus)*, and zebrafish *(Danio rerio)* genomes failed to reveal the presence of a colipase (Saele et al., 2010), which further supports the hypothesis that teleost fish appear primarily dependent on a bile salt dependent lipase (e.g., Murray et al., 2003).

Bile salts and alcohols act as detergents and serve to solubilize the lipid droplets before pancreatic lipase (aided by colipase) digests them. In mammals, bile salts are the major solutes secreted into bile, whereas in elasmobranchs and other fish, sulfated bile alcohols predominate. Hence, the alcohols are considered more primitive in comparison (Hagey et al., 2010). The major bile alcohol in elasmobranchs, scymnol sulfate, occurs at concentrations rivaling those of mammalian bile salts (Karlanganis et al., 1989). Enteral recovery of scymnol sulfate is high ($>$90%) in skates, although the fate of this absorbed bile salt is unknown (Fricker et al., 1997). It is possible it is recycled in the liver, or secreted via the kidney. The inhibition of cholesterol crystal formation by scymnol may be responsible for the absence of gallstones in elasmobranchs (Gilloteaux et al., 2013), although this deserves further investigation. Control of bile secretion appears to be via a gastrin- or cholecystokinin-like peptide (see Chapter 8) as in other vertebrates (Andrews and Young, 1988). However, acetylcholine did not affect gall bladder secretion of bile, despite being a potent stimulant in other vertebrates, suggesting a unique lack of neural control in elasmobranchs (Andrews and Young, 1988).

The activities of diverse nutrient catabolizing enzymes in elasmobranchs are similar to those seen in teleosts. For example, both trypsinogen activity, for protein digestion, (Zendzian and Barnard, 1967), and amylase activity, for starch and glycogen digestion, (Fig. 6.6C; Kuz'mina and Gelman, 1997) are only marginally elevated in elasmobranch GIT tissues. Enzymes involved in either carbohydrate or protein catabolism scale proportionally to dietary carbohydrates and proteins in teleost fish (Chakrabarti et al., 1995; Kuz'mina and Gelman, 1997). It remains to be seen if this is true for elasmobranchs. Ultimately, the observed increased levels of enzyme activity (Figs. 6.6A–C), together with a long passage time of digesta through the GIT, may represent adaptations to the shorter intestine found in elasmobranchs (excluding the spiral valve) in order to optimally digest their prey (Sibly, 1981). Additionally, any observed difference in GIT digestive physiology may represent trade-offs or adaptations to a variety of factors including feeding strategies (Fig. 6.1) and/or prey choice.

5. EFFECTS OF DIGESTION ON HOMEOSTASIS

Though a majority of research characterizes the underlying biochemical mechanisms, digestion exerts consequences on animal homeostasis and until recently, exploration of this phenomenon in elasmobranchs was limited. Specifically, studies examining the effect of digestion on nitrogen, ion and water, and acid/base balance in sharks have revealed interesting results.

Digestion produces ammonia through protein catabolism, both in the blood through tissues and organs and in the intestine. In ammoniotelic teleosts, digestion increases plasma ammonia levels (e.g., Bucking and Wood, 2008; Bucking et al., 2009). However, this dietary ammonia supports urea synthesis in ureotelic elasmobranchs, through the enzyme glutamine synthetase (GS). GS traps ammonia for production of urea in the ornithine-urea cycle. Interestingly, elasmobranchs exhibit a postprandial increase in plasma ammonia levels; however, it is blunted in size compared to teleosts (e.g., Wood et al., 2005; Kajimura et al., 2006 vs. Bucking and Wood, 2008; Bucking et al., 2009). After feeding, a transient increase in both urea and plasma trimethylamine oxide levels occurs (e.g., Kajimura et al., 2006; Wood et al., 2010), which has implications for osmoregulation (see Chapter 5). An increase in GS and other nitrogen metabolizing enzymes (both at the transcript and activity levels) is observed in dogfish following feeding (Kajimura et al., 2006; Walsh et al., 2006; Deck et al., 2013), revealing a potential benefit for osmoregulatory pathways in the ureotelic dogfish.

Indeed, Wood (2001) hypothesized that ammonia from digestion would be scavenged to contribute to urea production as dogfish sharks only feed sporadically and are thus nitrogen limited for urea production. Further supporting this hypothesis is evidence that rate of urea excretion does not change during digestion, indicating retention of nitrogen after feeding (Kajimura et al., 2006).

An increase in plasma urea (Kajimura et al., 2006; Wood et al., 2010) during digestion occurs in spite of a large secretion of urea into the GIT (Wood et al., 2007). Recent studies have potentially shown a complex urea-recycling program in the GIT. When fasting tissues were examined, urea was absorbed in all sections of the GIT except the intestine, where it was secreted (Liew et al., 2013). Upon feeding, there was a reversal from secretion to absorption in this section as well. This is in contrast to earlier studies on whole animal responses to feeding where a large net secretion of urea into the intestine was observed (Wood et al., 2007), possibly stemming from pancreatic and biliary secretions. The observed *in vitro* absorption then indicates an attempt by the intestine to scavenge the urea before elimination (Liew et al., 2013). Phloretin, ouabain, and sodium-free solutions inhibit urea transport in the dogfish intestine, indicating that urea is most likely dependent on several transport pathways; which supports the hypothesis that urea recycling is occurring in order to minimize nitrogen loss (Anderson et al., in press).

Little work exists examining ion and water transport pathways in the elasmobranch GIT. A recent study (Liew et al., 2013) has shown that the stomach was a site of sodium absorption following a meal, which is similar to work done in teleost fish (Bucking and Wood, 2007). In particular, there is little evidence for significant uptake of the divalent cations calcium and magnesium in any section of the GIT, whereas sodium, chloride, and water were absorbed in the intestine in both fasted and fed elasmobranchs (Wood et al., 2007; Anderson et al., 2007, 2010; Liew et al., 2013). In contrast, potassium was secreted in the intestine and was not affected by feeding (Anderson et al., 2007, 2010; Liew et al., 2013); however, this may be reflective of the *in vitro* technique itself as it contradicted earlier whole animal observations (Wood et al., 2007). Drinking, albeit at a low rate, has been suggested to occur in feeding elasmobranchs (Wood et al., 2007) possibly to help maintain osmotic balance between the GIT lumen and the plasma. The salt regulating function of the rectal gland is influenced by digestion, with an increase in NaCl secretion occurring post-meal ingestion (Wood et al., 2008, 2010). This response involves up-regulation of enzymatic activities (Walsh et al., 2006) but also transcriptomic responses (Deck et al., 2013), and presumably aids in eliminating an excess of salts absorbed during digestion.

Finally, digestion creates a systemic alkalinisation of the blood or an alkaline tide, in animals that employ gastric acid digestion (Niv and Fraser, 2002). Briefly, the production of a proton that is secreted into the stomach lumen for gastric acid formation necessitates the equimolar production of a base (HCO_3^-) that is secreted into the plasma to maintain pH balance in the cell. The previously observed addition of base to the blood of elasmobranchs during digestion verifies an alkaline tide is present (e.g., Wood et al., 2005). The role of the secretion of protons into the stomach lumen has been confirmed by inhibiting the activity of HKA in the stomach using a pharmacological agent, which results in the reduction of the excess base recorded in the plasma (Wood et al., 2009). This excess base results in an increase in base excretion to the water during feeding (Wood et al., 2005, 2007), and is attenuated by the reduction of gastric acid secretion (Wood et al., 2009). The relocation of H^+-ATPase transporters from cytoplasmic storage vesicles to the basolateral membrane of gill cells seen during digestion (Tresguerres et al., 2007; Roa et al., 2014) may drive the excretion of base to the water. Essentially, the excretion of protons into the blood may create an electrochemical gradient such that base (generated in equimolar concentrations by branchial carbonic anhydrase) is secreted to the environment.

6. FUTURE PERSPECTIVES

1. There is a need for physiological data to extend the utility of techniques such as SIA and QFASA. In particular, evidence of how elemental isotopes/fatty acids behave in biological systems is needed to avoid violating assumptions built into the analysis. Currently most evidence is from model organisms outside of Elasmobranchii, and there have been only limited investigations into chondrichthyes in particular. In order to take advantage of these techniques careful experimental studies on isotope and fatty acid tissue incorporation are needed in elasmobranchs. The confounding effects of environment (temperature, salinity, etc.); age; growth; dietary shifts; and nitrogen excretion can affect the calculated models and thus should be studied due to unique aspects of elasmobranch physiology.
2. Additional work is required regarding GIT enzyme activities in elasmobranchs. We know little about the enzyme activities in sharks, in stark contrast with the body of literature on teleost fish. Trypsin, elastase, carboxypeptidase, are just a few examples of digestive enzymes that are the potential subjects of future studies, in addition to continuing

work on lipase, colipase, and amylase discussed above. Species-specific differential expression, zonation along the GIT, and contrasts with other aquatic ectotherms are all potential areas of focus as well.

3. There is a need to know more about how the elasmobranch intestine responds to dietary shifts. Studies focusing on teleost fish show that dietary shifts are tolerated by some species with little compensation, while other species show tremendous plasticity. The response of elasmobranch intestines to dietary shifts is a fruitful area of experimental work, with little information currently existing. A large body of work on dietary prey composition shows how flexible some elasmobranch species are for the types of prey consumed. How this affects the GIT physiology may reveal how and why this variety of prey is tolerated by elasmobranchs.
4. Further work is needed to understand the interactions between whole animal and GIT physiology. For example, most elasmobranchs are ectotherms and the core temperature of these fish matches the surrounding water temperature unlike homeotherms. As ectotherms, temperature dependent processes in elasmobranchs (such as GIT enzyme activity and transport rates) will depend on the preferred water temperature that these animals inhabit, that is, the colder the water the animal is found in, the slower the reaction and transport rates. Some species of sharks generate body heat to elevate organ temperatures, and in some cases this endothermy appears to be a result of the retention of metabolic heat generated by digestion (e.g., Carey et al., 1981, 1985). In the lamnids studied so far, the temperature of the stomach (a good proxy measure for core body temperature) appears to be uniform over a small range of temperatures ($\sim$22–27°C). The gastric temperature is also elevated over, and independent from, ambient water temperatures (e.g., Carey et al., 1981; Goldman, 1997; Goldman et al., 2004; Béguer-Pon et al., 2012). The current assumption, that this elevated temperature enhances the rate of digestion and assimilation (Carey et al., 1981; Goldman, 1997), remains to be investigated thoroughly. Other aspects of whole animal physiology, such as ion or water balance, remain to be comparatively explored in elasmobranchs as well.

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